

Synchrony between fruit maturation and effective dispersers' foraging activity increases seed protection against seed predators

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The evolution of pollination and seed dispersal mutualisms is conditioned by the spatial and temporal co-occurrence of animals and plants. In the present study we explore the timing of seed release of a myrmecochorous plant (*Helleborus foetidus*) and ant activity in two populations in southern Spain during 2 consecutive years. The results indicate that fruit dehiscence and seed shedding occur mostly in the morning and correspond to the period of maximum foraging activity of the most effective ant dispersers. By contrast, ant species that do not transport seeds and/or that do not abound near the plants are active either before or after *H. foetidus* diaspores are released. Experimental analysis of diet preference for three kinds of food shows that effective ant dispersers are mostly scavengers that readily feed on insect corpses and sugars. Artificial seed depots suggest that seeds deposited on the ground out of the natural daily time window of diaspore releasing are not removed by ants and suffer strong predation by nocturnal rodents *Apodemus sylvaticus*. Nevertheless, important inter-annual variations in rodent populations cast doubts on their real importance as selection agents. We argue that traits allowing synchrony between seed presentation and effective partners may constitute a crucial pre-adaptation for the evolution of plant–animal mutualisms involving numerous animal partners.

Keywords: seed dispersal; myrmecochory; preadaptation; ant; phenology; mutualism

1. INTRODUCTION

The evolution of pollination and seed dispersal mutualisms has been debated since Darwin, Wallace and Müller (Thompson 1994). To resolve the major obstacle that immobility causes for gene dispersal, many plants rely on animals to transport their pollen or seeds in exchange for food rewards. Multiple examples show that pollinators visit flowers primarily for the nectar they secrete (Faegri & van der Pijl 1979; Pellmyr 2002) and that the main function of the pulp of fleshy fruits is to attract bird or mammal dispersers (Jordano 1992; Herrera 2002). The seeds of more than 3000 myrmecochorous plants also possess a lipid-rich food reward, the elaiosome, which triggers dispersal by ants (Mayer *et al.* 2005). However, these rewards also attract less effective mutualists and cheaters that try to take the food without providing an adequate service. Current investigations seek to determine whether structures and mechanisms that increase visits by the most effective mutualists derive from selective pressures exerted by animals or constitute pre-adaptations favouring the evolution of mutualisms (Irwin *et al.* 2004; Giladi 2006).

Presenting diaspores and their associated rewards at the right moment may greatly enhance the probability of being removed by an effective partner. For example, the seasonal timing of fleshy fruit ripening in deciduous temperate forests and Mediterranean scrublands occurs in late summer and early winter, respectively, which coincides

with the presence of migrating frugivores in these areas (Stiles 1980; Herrera 1984). On the other hand, many myrmecochores mature in spring and shed seeds when ant foraging activity is high and food (e.g. dead insects) is still scarce which augments seed removal (Thompson 1981; Ohkawara *et al.* 1997; Oberrath & Böhning-Gaese 2002; Guitián & Garrido 2006). Although seasonal correspondence between fruit ripening and disperser abundance or foraging activity is obviously beneficial for plants, it does not necessarily result from selection exerted by mutualists on plant phenology (Herrera 1995, 2002). In the case of bird-dispersed plants, alternative hypotheses such as selection due to abiotic factors acting independently on both plants and animals may explain the observed temporal correspondence (Debussche & Isenmann 1992; Fuentes 1992). For ant-dispersed plants, the 'disperser selection pressure' hypothesis is contradicted by the observation that the probability of seed removal by ants sometimes increases after the seasonal peak of seed release (Ruhren & Dudash 1996).

Many ant-dispersed plants produce dehiscing capsules or carpels that release seeds at a particular moment of the day (Turnbull & Culver 1983; Espadaler & Gómez 1996; Narbona *et al.* 2005). In the Rocky Mountains of Colorado, at approximately 3000 m elevation, the capsules of *Viola nuttallii* open mostly in the morning, which coincides with the hour of maximum foraging activity of its main ant dispersers in the area, *Myrmica discontinua* and *Formica podzolica* (Turnbull & Culver 1983). It was suggested that delivering seeds in the morning may give ants enough time to remove them before they are predated by nocturnal rodents (Turnbull & Culver 1983;

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Gibson 1993; Ruhren & Dudash 1996; Giladi 2006). In the present study we aim to test this hypothesis by going one step further, using a system where the ant community is more diverse than those previously studied and includes species that differ broadly in their dispersal effectiveness.

Helleborus foetidus is a myrmecochore that grows in mountainous regions of Western Europe, including Mediterranean habitats where ant communities are frequently composed of 10–20 species (Retana & Cerdá 2000; Garrido *et al.* 2002). In theory, the effectiveness of dispersal by ants may vary as a function of (i) the probability of their finding *H. foetidus* elaiosome-bearing seeds, which depends on their relative abundance where the plant grows and (ii) their propensity to remove diaspores when they find them. Studies conducted with *H. foetidus* and other model systems indicate that while some ant species rapidly retrieve myrmecochore diaspores to their nest, others only chew the elaiosome *in situ* or simply ignore them (Horvitz 1981; Andersen 1988; Christian 2001; Garrido *et al.* 2002; Boulay *et al.* 2007). Since non-ant-removed *H. foetidus* seeds are exposed to predation by field mice *Apodemus sylvaticus* (Fedriani *et al.* 2004), we hypothesized that this plant may derive an advantage from presenting its seeds at a particular time of day when effective dispersers are active. This may occur if ants directly exert a selection pressure on the timing of seed shedding or if seed shedding depends on environmental cues that also shape the ant community.

Ant daily rhythm of foraging activity is often constrained by complex interactions between interspecific competition, temperature resistance and pattern of food availability (Cros *et al.* 1997; Sanders & Gordon 2000; Albrecht & Gotelli 2001). In particular, in Mediterranean habitats, ant species commonly segregate according to their tolerance to high temperatures. To alleviate interspecific competition, some species trade off the risk of dying from elevated temperature with better foraging performances (Cerdá *et al.* 1997, 1998). In turn, daily variations in the availability of certain categories of food may determine the feeding preferences of species that co-occur at the same time. For example, in open grassland, Retana *et al.* (1991) observed that a major proportion of ants foraging at the warmest hours of the day were scavengers. Several studies have suggested that, because elaiosomes contain long-chain fatty acids that are also present in insect corpses, myrmecochory should involve mostly generalist scavenger ants (Marshall *et al.* 1979; Skidmore & Heithaus 1988; Hughes *et al.* 1994; Boulay *et al.* 2006). We therefore expected that plants would release their seeds when a high proportion of scavenger ants were active.

In order to test whether the daily rhythm of seed shedding coincides with that of more effective ant dispersers and eventually reduces predation by rodents, we determined (i) the daily rhythm of *H. foetidus* seed release in relation to temperature variations in two Mediterranean populations during 2 consecutive years, (ii) the pattern of foraging activity of the various ant species living in these populations, (iii) their dispersal effectiveness, taking into account both their local abundance and propensity to remove *H. foetidus* diaspores, (iv) the potential relation between ant dispersal effectiveness and foraging activity during the peak of seed release, (v) the relation between dispersal effectiveness and diet

preference, and (vi) the consequence of synchrony between seed shedding and disperser activity for seed predation by rodents.

2. MATERIAL AND METHODS

(a) Study system and site

Helleborus foetidus is a perennial herb relatively common in the understory of deciduous and mixed forests of the Iberian Peninsula. After several years of vegetative growth, it produces one to three reproductive ramets, each composed of 25–100 apocarpous flowers. Carpels open gradually between late May and mid-June and release 10–12 elaiosome-bearing seeds each. Ants remove the diaspores on the ground or visit the plants to detach the diaspores from dehiscing carpels and transport them to their nest. As in many myrmecochorous plants, the seed of *H. foetidus* possesses a strong tegument that limits seed predation by ants (Rodgers 1998; Boulay *et al.* 2005) but does not prevent predation by rodents (Fedriani 2005; Fedriani & Boulay 2006).

The study was conducted during the spring and the summer of 2004 and 2005 in two southern Spanish localities approximately 100 km apart: Sierra de Cabra (37°30.66' N, 04°21.21' W; 960–1010 m elevation; Cabra hereafter) and Sierra de Grazalema (36°47.11' N, 05°25.09' W; 730–750 m elevation; Grazalema hereafter). Both populations were surrounded by scarce trees (e.g. *Quercus lusitanica*, *Quercus ilex*, *Acer monspessulanus*) and low understory. All surveys and experiments were conducted on days with representative weather for the season.

(b) Timing of carpel dehiscence

We studied the rate of carpel dehiscence on 30 focal plants per population and year. These plants were separated by at least 5 m. The total number of carpels they produced was counted during a preliminary survey conducted at the beginning of May of both years, approximately three weeks before diaspore release. The timing of carpel dehiscence was surveyed in June by counting the number of closed carpels on each plant every 2 hours from 08.00 (local time; approx. 1 hour after sunrise) to 20.00 (local time; approx. 1 hour before sunset) and at 08.00 the following day. Temperature was measured on the plant, at approximately 30 cm from the ground, to the nearest 0.5°C during each census. The rate of carpel opening was calculated as the number of carpels that opened out between two consecutive censuses expressed in percentage of the number of carpels that opened out throughout the 24 hours survey, divided by the time between censuses.

(c) Ant and mice abundance

Ant and mouse abundances were sampled in 2004 and in 2005 in both populations. To limit interferences with other surveys, two samplings were conducted per year approximately three weeks before and after the peak of seed release (mid-spring and early summer sampling periods, respectively). The ants that visited focal plants were sampled by placing two pitfall traps (4 cm in diameter, 7 cm deep plastic cup filled with water and soap) approximately 1 m apart on opposite sides of each shoot during 24 hours. The biological material of the two traps was pooled and considered as one single sampling unit. The relative abundance of an ant species was calculated as the proportion of sampling units in which at least one worker was encountered.

Field mice were captured during two to three consecutive nights per sampling period (mid-spring and early summer) of each year (total: five to six nights per year and population). Thirty pairs of traps were placed approximately 1 m apart on opposite sides of each focal plant. Twenty-eight additional pairs of traps were used per sampling and were placed near reproductive plants in the same area and separated by at least 5 m. Each pair of traps was composed of one Ugglan-type trap that closes by gravity and one 'Hipolito-type' trap (Carro *et al.* 2007) that closes when the animal tries to remove the bait (peanut butter). All traps were checked at dawn and dusk. Captured mice were sexed, marked by toe clipping and released at the spot of capture.

(d) Ant daily rhythm of activity and diet preference

Ant daily activity and diet preference were studied at the beginning of June 2004 and 2005 in both populations, a day after the survey of carpel dehiscence. Food baits composed of sliced meal worms (*Tenebrio molitor*), sesame seeds and honey were placed at 07.30 at approximately 1 m from the focal plants. Food items were delivered separately in 3 cm diameter Petri dishes. Ant presence in the baits was surveyed every 2 hours from 08.00 to 20.00. Food baits were replenished after each census. The rhythm of activity was estimated from the number of occupied baits per time interval divided by the total number of occupied baits from 08.00 to 20.00. Diet preference of each species was established from the frequency of observations of at least one worker on the three different types of food bait. The measure of ant activity was unlikely to have been affected by the estimation of ant abundance that was done approximately three weeks before.

(e) Diaspore removal

The study of diaspore removal was conducted at the beginning of June 2004 and 2005 a day after the survey of ant rhythm of activity. At 08.00, pairs of Petri dishes containing eight *H. foetidus* diaspores collected the day before in the area of study were placed at approximately 1 m from the focal plants. A wire mesh allowing free ant passage but preventing mouse access covered one of the two seed depots (ants only treatment). The other depot was open to allow both ant and rodent accesses (ant + mouse treatment). Every 2 hour from 10.00 to 20.00, we counted the number of remaining seeds in each depot. The seeds were systematically changed after each census. At 20.00, 48 diaspores were placed in each depot and the number of seeds removed overnight was surveyed at 08.00 the following day (i.e. 12 hour later). The rate of seed removal was calculated as the proportion of removed seeds divided by the time between two consecutive surveys.

Whenever ants were observed in depots that still contained seeds, we recorded whether they were removing them or not. The propensity of an ant species to remove *H. foetidus* diaspores was calculated as the number of times at least one worker of this species was observed removing a diaspore divided by the number of times it was observed in the depots.

(f) Data analyses

Generalized linear model (GLM) analyses were conducted with the GENMOD procedure for SAS v. 9.1. Simplification of full models that initially included more than three factors was achieved by backward elimination of non-significant ($\alpha < 0.05$) interactions and main factors. Differences between

levels were estimated from least square mean difference using the LSMEANS statement. The number of carpels produced by the focal plants was compared between years and populations with a GLM with the Poisson distribution and log link function. The rate of carpel opening was analysed with a GLM with the negative binomial error distribution and log link function. The full model that was initially run included time interval (repeated factor), population, year, the average temperature during the time interval, the variation of temperature during the time interval and their interactions. The number of mouse captures divided by the number of nights of trapping was fitted with the negative binomial error distribution and log link function to population, year and their interaction. The Pearson χ^2 -test was used to test deviations of mouse sex ratio from 1 : 1 and differences of sex ratio between populations and years.

Ant dispersal effectiveness was calculated for each species as the square root of the product of its relative abundance in a population by its propensity to remove *H. foetidus* diaspores. The significance of the association between ant species dispersal effectiveness and their proportion of daily foraging activity during the peak of diaspore release was tested using Pearson correlation. We also tested the possible relation between ant diet preference and dispersal effectiveness. To that end we first conducted a cluster analysis with Ward's method to identify consumer categories from the proportion of observations of each species on worms, honey and sesame seed bait. These categories were confirmed by MANOVA. Then, we compared ant dispersal effectiveness between consumer categories using the median test.

Finally, the probability of diaspore removal from the depots was fitted with the binomial error distribution and logit link function. The full model included time interval (repeated factor), year, population and treatment (ant only versus ant + mice) and their interactions. All estimates are means \pm s.e.

3. RESULTS

(a) Daily rhythm of seed release

The focal plants produced 77 ± 5 and 74 ± 7 carpels, at Cabra and Grazalema, respectively (GLM, population: $\chi^2_1 = 0.17$, $p = 0.679$). In both populations, the plants were bigger in 2004 than in 2005 (99 ± 7 versus 53 ± 4 , respectively; GLM, year: $\chi^2_1 = 31.92$, $p < 0.001$; year \times population: $\chi^2_1 = 1.01$, $p = 0.314$). Throughout the day, the rate at which carpels opened out varied greatly (figure 1; GLM, time interval: $\chi^2_6 = 69.4$, $p < 0.001$) and differed slightly between populations (GLM, population \times time interval: $\chi^2_6 = 19.8$, d.f. = 6, $p = 0.006$). In both years, the focal plants shed most of their seeds between 12.00 and 14.00 at Cabra and between 10.00 and 12.00 at Grazalema. Later in the afternoon, the rate of carpel opening decreased progressively and approached zero during the night. Carpel opening co-varied with the variation of temperature during the time interval (figure 1; GLM, variation of temperature: $\chi^2_1 = 4.33$, $p = 0.037$), which was maximum between 12.00 and 14.00 at Cabra and between 10.00 and 12.00 at Grazalema. All other studied variables, including the average temperature during the time interval, did not affect significantly the rate of carpel opening.

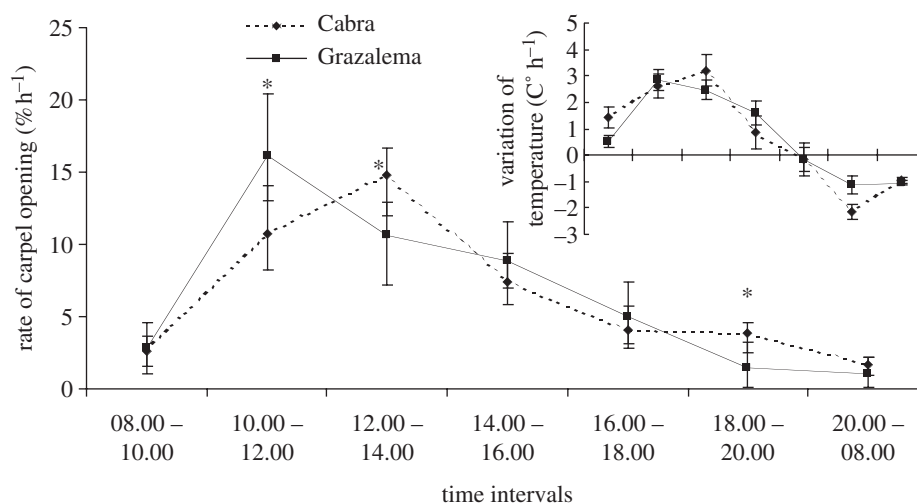


Figure 1. Rate of carpel opening throughout the day at Cabra and Grazelema (model-adjusted means \pm 95% CI). Asterisk indicate significant differences between populations for a given time interval. The inserted graph shows the variation of temperature occurring during the time intervals.

(b) Ant abundance, dispersal effectiveness and rhythm of activity

Pitfall traps revealed the occurrence of 14 and 13 ant taxa at Cabra and Grazelema, respectively, of which eight were common to both populations (see the electronic supplementary material for species abundance, behaviour and diet preference). In both populations, the most frequent species was *Aphaenogaster senilis*, which appeared in 70 and 87.5% of the sampling units at Cabra and Grazelema, respectively. This species was also the most prompt in removing diaspores, as workers removed diaspores 75% of the times they were observed in the *H. foetidus* depots. It was therefore shown to be a very effective disperser of *H. foetidus* in both populations. Some species, such as *Camponotus lateralis*, were relatively abundant but behavioural observations indicated they were very ineffective dispersers that most often chewed the elaiosome without removing the diaspore. The propensity of other species, such as *Aphaenogaster gibbosa* to remove diaspores was relatively high but they were too rare to constitute effective dispersers. Finally, species such as *Tetramorium caespitum* and *Tetramorium impurum* were neither abundant around the plants nor prompt to remove diaspores, their effectiveness therefore being close to zero.

In both populations, ant dispersal effectiveness was significantly correlated with the percentage of activity during the time period of maximum seed release (figure 2; Pearson's correlation, Cabra: $r^2=0.32$, d.f. = 14, $t=2.40$, $p=0.034$; Grazelema: $r^2=0.37$, d.f. = 11, $t=2.32$, $p=0.045$). The most effective dispersers were species that were mostly active when diaspore availability was the highest. By contrast, poor dispersers were either active before or after the peak of seed shedding in the respective population. This result is well exemplified in figure 3, which shows that the two best dispersers at Cabra (*A. senilis* and *Camponotus cruentatus*) were very active between 12.00 and 14.00, when seeds were released, while the two worst dispersers (*C. sylvaticus* and *Tapinoma nigerrimum*) were mostly active at dawn and dusk.

We were able to determine experimentally the feeding preference of all but three species that were too rare (*Stenamma orousetti*, *Crematogaster auberti* and *Crematogaster sordidula*). Cluster analysis conducted on the

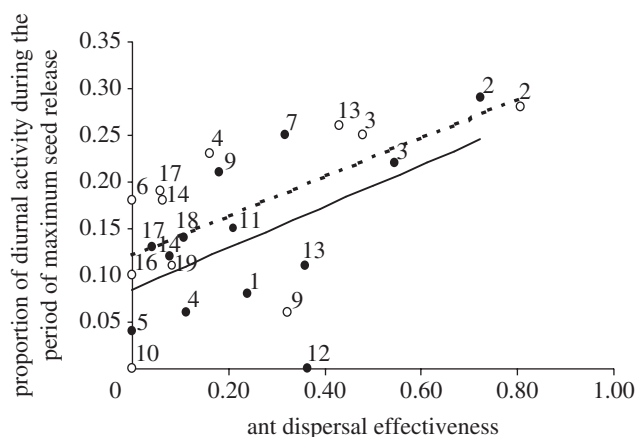


Figure 2. Relation between ant dispersal effectiveness and proportion of diurnal activity during the period of maximum seed release at Grazelema (10.00–12.00, open symbols; fit: dash line) and Cabra (12.00–14.00, closed symbols; fit: solid line). The numbers refer to ant species as follows: 1, *Aphaenogaster gibbosa*; 2, *Aphaenogaster senilis*; 3, *Camponotus cruentatus*; 4, *Camponotus lateralis*; 5, *Camponotus sylvaticus*; 6, *Cataglyphis rosenhaueri*; 7, *Cataglyphis velox*; 8, *Crematogaster auberti*; 9, *Crematogaster scutellaris*; 10, *Crematogaster sordidula*; 11, *Formica subrufa*; 12, *Messor structor*; 13, *Pheidole pallidula*; 14, *Plagiolepis pygmaea*; 15, *Stenamma orousetti*; 16, *Tapinoma nigerrimum*; 17, *Temnothorax* spp.; 18, *Tetramorium caespitum*; 19, *Tetramorium impurum*.

percentages of occupation of the baits allowed us to identify four consumer categories which were further confirmed by MANOVA ($F_{6,22}=18.0$, $p<0.001$). Two groups, N_1 and N_2 , were mostly observed feeding on honey, but the former occasionally fed on worms and the latter on sesame seeds. The third group (O) was composed of omnivorous ants that were observed on the three kinds of food. The fourth group was composed of two granivorous species. The four consumer categories differed with respect to their dispersal effectiveness (median test: $\chi^2_1=11.2$, $p=0.011$), although no pairwise comparisons proved to be significant, probably because non-parametric tests lacked power after the Bonferroni procedure. Nevertheless, dispersal effectiveness tended to be higher among omnivorous species than in other consumer categories.

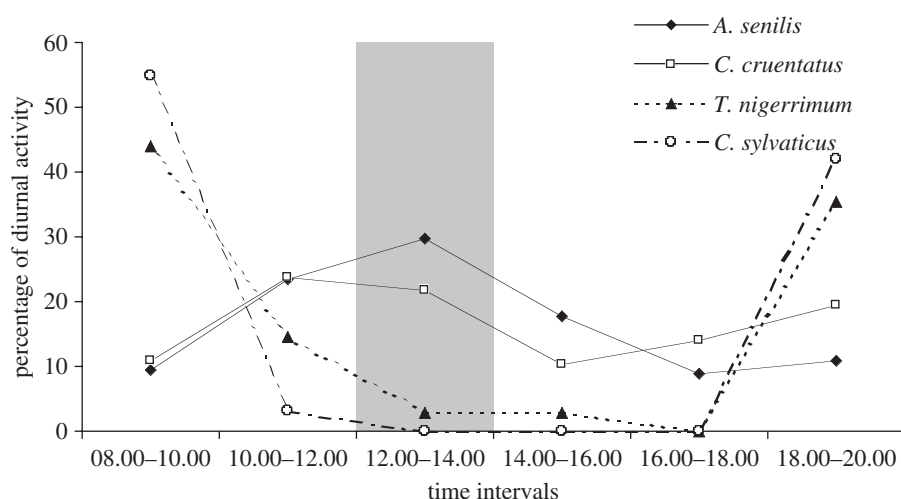


Figure 3. Activity rhythm of four ant species at Cabra. Values are averages of 2004 and 2005. *Aphaenogaster senilis* and *C. cruentatus* are the two best dispersers while *T. nigerrimum* and *C. sylvaticus* are the two worst dispersers. The area in grey represents the period of maximum diaspore availability in this population.

(c) Mouse abundance

Field mice *A. sylvaticus* L. are completely nocturnal and all captures occurred during the night. In 2004, we carried out 65 and 38 captures in five and six nights of trapping at Cabra and Grazalema, respectively. By contrast, in 2005 only 10 and 2 captures were carried out during six nights in both populations. Hence, the probability of capture was significantly lower in 2005 than in 2004 (GLM, year: $\chi^2_1 = 13.12$, $p < 0.001$), and at Grazalema than at Cabra (GLM, population: $\chi^2_1 = 97.72$, $p < 0.001$). The interaction between population and year was not significant (GLM: $\chi^2_1 = 1.05$, $p = 0.306$). In total, 79 individuals were captured, of which 25 individuals were captured more than once. The sex ratio (38 males and 41 females) was not significantly different from 1 : 1 (Pearson's χ^2 : $\chi^2_1 = 0.11$, $p = 0.737$), nor was it different between populations (Pearson's χ^2 : $\chi^2_1 = 0.96$, $p = 0.327$) and years (Pearson's χ^2 : $\chi^2_1 = 0.11$, $p = 0.743$).

(d) Diaspore removal by ants and mice

Diaspore removal varied significantly between time intervals and was maximum between 10.00 and 14.00 (figure 4; GLM, time interval: $\chi^2_6 = 52.97$, $p < 0.001$). Diaspores that were deposited earlier (at 08.00) and later (from 14.00 to 20.00) were less readily removed. During the day, diaspore removal did not differ between rodent-excluded and -allowed depots. However, during the night, diaspore removal was lower in rodent-excluded depots (GLM, time interval \times treatment: $\chi^2_7 = 14.98$, $p < 0.040$, difference of least square means among treatments between 20.00 and 08.00: $\chi^2_1 = 7.24$, $p = 0.007$). Finally, the significant three-way interaction between time interval, treatment and year (GLM: $\chi^2_{14} = 28.93$, $p = 0.011$) indicated that this effect was more pronounced in 2004 than in 2005. In 2004 between 08.00 and 10.00, diaspore removal was higher in depots that were accessible to ants only than in depots accessible to ants and mice. No other effect was significant.

4. DISCUSSION

In the study, they conducted more than two decades ago, Turnbull & Culver (1983) pointed out that in the Rocky Mountains *V. nuttallii* released most seeds at the hour of

the day with highest ant foraging activity. Our own results demonstrate that *H. foetidus* also releases its seeds in a marked daily rhythm, which favours seed discovery and dispersal by ants. A major difference between both studies, however, is that the Mediterranean ant communities of Cabra and Cazorla are much richer than the alpine community studied by Turnbull and Culver and include species with very diverse dispersal effectiveness. This allowed us to demonstrate that the daily peak of *H. foetidus* seed presentation occurs precisely when the most effective ant dispersers are active. *Helleborus foetidus* mostly releases seeds in late morning to early afternoon, which coincides with the very moment of the highest foraging activity of *A. senilis* and *C. cruentatus*. Both species are omnipresent around the plants and are able to rapidly discover *H. foetidus* diaspores. Moreover, once they discover a diaspore they almost systematically remove it to their nest instead of feeding on the elaiosome *in situ* ('active ants on dispersal' sensu Baiges et al. 1991). By contrast, ineffective dispersers, such as *C. sylvaticus* and *T. nigerrimum* ('non-active ants on dispersal' sensu Baiges et al. 1991), were not active at all when reproductive shoots shed seeds. Between these extremes, ants with intermediate dispersal effectiveness such as *Pheidole pallidula* and *Cataglyphis velox* ('active/non-active ants on dispersal' sensu Baiges et al. 1991) were already decreasing foraging or not yet fully active, at the time of highest seed release.

By measuring ant activity on food baits that were maintained constant during 12 hours independently of the natural availability of myrmecochorous seeds, we can discard the hypothesis that the activity pattern of seed-removing ant simply followed the temporal dynamics of seed release. A drawback of this method is to overestimate the activity range of dominant species that monopolize food baits with respect to solitary foraging species. Nevertheless, we believe this effect may be reduced since even when hundreds of *P. pallidula* or *T. nigerrimum* were recruited at baits, they never completely deter behaviourally subordinate species such as *A. senilis* from remaining around an appetitive food source.

The results of the depot experiment suggest that synchrony between seed release and disperser activity may affect seed survival when predator population is high

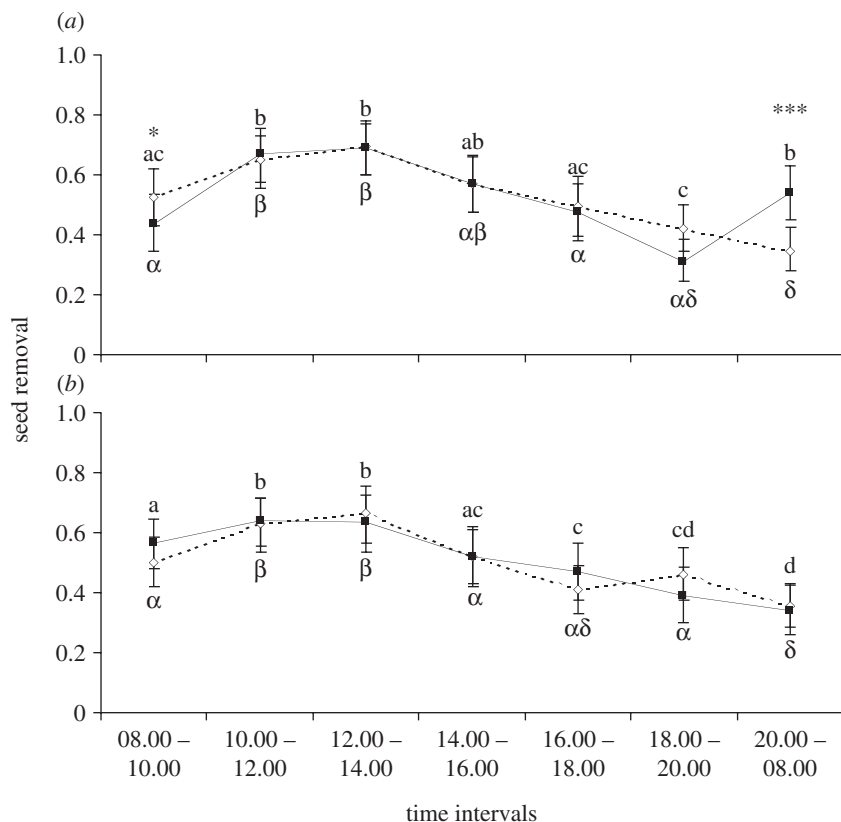


Figure 4. Proportion of *H. foetidus* diaspores removed from the artificial depots in (a) 2004 and (b) 2005. Values are model-adjusted means \pm 95% CI. Different Roman letters denote significant differences between time intervals for depots accessible to ants and mice (solid line). Different Greek letters denote significant differences between time intervals for depots accessible to ants only (dash line). Stars denote significant differences between treatment for a given time interval and year (* p < 0.05; *** p < 0.001).

(e.g. in 2004). In effect, seeds deposited at the time of maximum seed release were rapidly discovered and removed by effective dispersers. On the contrary, those delivered outside this time window were more likely to be discovered by ants that only consume elaiosomes, thus reducing their attractiveness to effective dispersers (Garrido *et al.* 2002; Gorb & Gorb 2003). As a consequence, seeds that were deposited outside the natural peak of seed release were more likely to remain exposed until nocturnal predators became active. Field mice are not known to cache *H. foetidus* diaspores (Fedriani & Boulay 2006) and probably consumed all the seeds they removed in 2004. The fate of seeds removed by effective dispersers before rodent activity is unknown and may depend on the ant species. Most species may simply remove the elaiosome and discard the intact seed on the refuse pile or in a specific nest chamber with other nutrient-rich food remains, which may favour germination. Even strictly granivorous ants such as *Messor structor* may not be able to remove the strong tegument that protects the *H. foetidus* embryo and may mostly feed on the elaiosome (Rodgers 1998; Boulay *et al.* 2005).

Although the previous result reinforces the idea that myrmecochory provides protection against granivores through seed burying in ant nests (Turnbull & Culver 1983; Gibson 1993; Ruhren & Dudash 1996; Boyd 2001; Christian & Stanton 2004; Manzaneda *et al.* 2005), major inter-annual variations of mouse abundance cast doubts on the role of granivory as a selection pressure affecting the timing of seed release. In our study, mouse populations at both sites and seed removal from rodent-allowed depots decreased astonishingly in 2005, probably owing to a lack

of food during a dry winter. This suggests that shoots of *H. foetidus* that live a few years may experience several reproductive events during which predator-induced selection varies greatly. Moreover, large-scale geographical variations in mouse populations may reduce the importance of granivory on plant fitness (Fedriani *et al.* 2004; Manzaneda *et al.* 2005). As a consequence, the correspondence between the timing of carpel dehiscence and the activity of the most effective dispersers may not necessarily result from predator-induced selection.

Although carpel dehiscence by loss of cell turgescence may be governed primarily by genetically determined hormonal factors, our results suggest that it is also affected by differential conditions of temperature. Hence, a rapid increase of ambient temperature preceded *H. foetidus* carpel dehiscence at both study sites. In the laboratory, carpel opening also increases with temperature (X. Cerdá 2002, unpublished data). A similar phenomenon was observed in other plants producing dehiscing capsules (Giliberto *et al.* 1980). In our study, morning temperature augmentation occurred earlier at Grazalema than at Cabra, perhaps because the latter population is located at a higher elevation, and probably induced carpel dehiscence to occur later at Cabra than at Grazalema. Interestingly, in spite of this difference, seed shedding coincided with effective disperser activity at the two localities, suggesting that both may be influenced by the same temperature-related factors.

Several studies have shown that in Mediterranean habitats temperature has an important structuring effect on ant communities (Cerdá *et al.* 1997, 1998). During the hot season, a number of dominant species that cannot

tolerate elevated temperatures tend to displace their foraging activity towards dusk and dawn. More temperature-tolerant species take advantage of reduced intraspecific competition and forage during the warmer time window (Cros *et al.* 1997). Species foraging at relatively high temperatures may share a set of traits that also make them relatively effective dispersers. For example, it was suggested that relatively heat-tolerant species should have a broader feeding spectrum to remove scarce food items (Retana *et al.* 1991). In our study, four species (*P. pallidula*, *A. senilis*, *C. cruentatus* and *C. velox*) were categorized with a particularly broad feeding preference. These scavenger species, which consumed honey, dead insects and occasionally sesame seeds, were also relatively effective dispersers of *H. foetidus*. This apparent relationship may be explained by the resemblance between the lipid composition of elaiosomes and that of insect prey, which apparently constitute an important part of their diet (Hughes *et al.* 1994; Boulay *et al.* 2006). By contrast, less effective species that foraged at cooler temperature were more often recorded on honey or seeds than on dead insects. This confirms previous observations, suggesting that ants involved in myrmecochories are non-specialist consumers with very broad feeding spectrum (Beattie & Hughes 2002; Boulay *et al.* 2005).

Other myrmecochores also release their seeds mostly in the morning when ant activity is high (*V. nuttallii*, Turnbull & Culver 1983; *Euphorbia characias*, Espadaler & Gómez 1996; *Melampyrum lineare* and *Melampyrum silvaticum*, Gibson 1993). Our results with *H. foetidus* demonstrate that this correspondence is finely tuned and clearly augments seed removal by efficient seed dispersers. However, evidence that such an adaptation is a consequence of selection pressure by dispersers and predators on plants is still poor, and a more parsimonious explanation may hold, for example, that both ants and plants respond to temperature-related abiotic factors. This does not mean that synchrony of disperser activity and seed presentation is casual and neutral in evolutionary terms. We argue that morphological and physiological traits allowing temporal and spatial co-occurrence of potential ant dispersers and seeds may consist of pre-adaptations that were crucial for other traits (e.g. elaiosome chemistry) to evolve, irrespective of the specific advantage procured by myrmecochory. This illustrates a recent call to identify general rules determining ant dispersal effectiveness and mechanisms allowing plants to attract the most effective dispersers Giladi (2006). In addition, we suggest that many other apparent phenologic adaptations that at first sight augment dispersal and pollination by animals may find their origin before the evolution of such mutualisms. This may be particularly true if the reward can attract many animal species, of which only a fraction provides the service. In the future, comparative analyses accounting for phylogeny could help to test this hypothesis.

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